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Improvement of Salinity Tolerance Indices and Regulation of Na^+ and K^+ Homeostasis in Hashemi Rice Mutants (*Oryza sativa* L.)

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ABSTRACT

In this study, we evaluated the effectiveness of gene expression changes on ion homeostasis comprising Salt Overly Sensitive (SOS1) and vacuolar Na+/H+ antiporter (NHX1) along with ion and proline content measurement in Hashemi rice mutants at Rice Research Institute of Iran, in 2018-2019. Tolerant mutant genotypes (em_4hs290 and em_4hs84) along with Hashemi parent cultivar, IR28 (sensitive), and FL478 (tolerant) seedlings were treated with 100 mM NaCl. Based on the results of growth indices, the seedling length of Hashemi cultivar and IR28 decreased considerably, about 44.7, and 44.2% reduction compared to the control, and the leaves progressively yellowed. Results showed that proline content and K^+ and K^+/Na^+ ratio increased about ~2–3-fold in the tolerant genotypes than in the susceptible ones. Also, the overall amount of the *OsNHX1* and *SOS1* expression increased in tolerant genotypes compared to the susceptible ones. Accordingly, the compatible solute accumulation significantly advanced, resulting in improvement of ionic homeostasis and, probably, suppressed the stress. Moreover, the variable pattern of gene expression in the two salt-tolerant mutants (em_4hs290 and em_4hs84) and Hashemi parent showed that the induced mutation could increase the salt-tolerant in mutant genotypes through ionic and osmotic homeostasis. Generally, these tolerant mutant genotypes could be applied to develop salt-tolerant varieties in rice breeding programs, which can lead to production sustainability.

Keywords: Gene expression, Mutation, Stress Index.

INTRODUCTION

Increased food production is undeniably necessary to meet the nutrient needs of the growing world population to 9 billion in the 2050s. Salt stress, as one of the most intense environmental problems, influences the potential of plant production and induces significant crop loss worldwide (Zhang *et al*., 2018). Previous studies show that climate changes and inappropriate irrigation practices enhance salt accumulation in soil (Pitman and Läuchli, 2002; Roy *et al*., 2014), and lead to incurring significant costs of approximately $12 billion per year globally (Pitman and Läuchli, 2002). Identifying traits related to salinity tolerance is required to improve this trait and high-yielding genotypes (Munns and Tester, 2008). Most cultivated rice varieties are susceptible to salinity stress; their salinity threshold is three dSm^{-1} (Chinnusamy *et al*., 2005; Munns, 2005). Despite numerous attempts and various strategies including genetic engineering to develop salinity tolerance in rice, the achievements are relatively moderate (Hoang *et al*., 2016; Royan *et al*., 2023; Ramzi *et al*., 2020) So, the breeding cultivars of salinity tolerant with the ability to grow in saline soils is

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critical for sustainable agriculture and food security (Zhang *et al*., 2022). Though achievement of salinity-tolerant rice cultivars is time-consuming (taking at least 6 to 7 years), laborious, and incompetent through traditional breeding programs (Sun *et al*., 2017; Wang *et al*., 2019). Therefore, mutation breeding approach is a fast and critical method for creating genetic diversity in favorite traits (Ahloowalia *et al*., 2004), containing the development of tolerant cultivars to biotic and abiotic stress and agronomic traits improvement (Masoabi *et al*., 2018). In many research, salinity-tolerant rice mutants were created using a combination of induction mutations and in vitro selection (Huong *et al*., 2020; Yunita *et al*., 2020; Zhang *et al*., 2019).

Plants have developed several mechanisms to tolerate salt stress. The most effective mechanism of salinity tolerance is selective regulation of Na^+ uptake and efflux systems with limitation in sodium ions (Na^+) admission into the cytosol (Ji *et al*., 2013; Zhu, 2003). Because of resemblances in ionic characteristics, Na^+ can contest with and absorb through potassium ions (K^+) uptake systems. Na^+ efflux from roots and Na^+ sequestration within vacuoles would occur if cytosolic Na^+ levels in plants decrease (Craig Plett and Møller, 2010). Moreover, proline, as an important osmolyte, plays in the modulation osmotic potential of cells under drought and salinity stresses in some plants (Bagheri *et al*., 2023). Accumulation of proline could enhance plant salinity tolerance by decreasing the destructive effect of salinity. Many studies demonstrated that the novel salt-tolerant rice genotype increased proline content under salt stress (Nahar *et al*., 2022, 2023; Koc *et al*., 2024). The plants salt tolerance is a complicated trait containing several physiological and biochemical mechanisms (Ganie *et al*., 2019; Rasel *et al*., 2021).

Both Salt Overlap Sensitive genes in rice (OsSOS1/OsNHX7) and Arabidopsis (AtSOS1/AtNHX7) encode a plasma membrane Na^+/H^+ antiporter, which has principal roles in Na^+ extrusion in the roots under salinity conditions (Chinnusamy *et al*., 2005; Ding and Zhu, 1997). Therefore, Na^+/H^+ antiporters of *SOS1* and *NHX1* have contained the principal role of Na^+ exclusion and sequestration, to decrease salinity toxicity in the plant. Therefore, Na^+ uptake in plants is done using several ion channels and carrier-type transporters, which have been identified. The cation/H^+ exchange through membranes is catalyzed by identified NHX-type antiporters (Bassil and Blumwald, 2014; Jiang *et al*., 2010). The compartmenting of Na^+ ions into the vacuole is mediated conventionally by the function of tonoplast (vacuole membrane) localized NHX-type.

Six family members of rice NHX-type antiporter were recognized as associated with three subclasses with various cellular localizations: SOS1 is located in the plasma membrane (Martínez-Atienza *et al*., 2007) and five other intracellular members comprising *OsNHX1* up to *OsNHX4* and *OsNHX5* are located in the tonoplast and prevacuolar compartment, respectively (Fukuda *et al*., 2011; Fukuda *et al*., 1999). Previous studies revealed that some plant species advanced salt and drought stress tolerance via *NHX1* overexpression (Xue *et al*., 2004; Xiao *et al*., 2009; Zhang and Blumwald, 2001; Liu *et al*., 2010; Ohta *et al*., 2002) and K^+ homeostasis effectively adjusts through *NHX1* and *NHX2* (Andrés *et al*., 2014; Barragán *et al*., 2012). The plasma membrane Na^+/H^+ antiporter, Salt Overly Sensitive 1 (SOS1), is the most characteristic Na^+ efflux protein in plants (Shi *et al*., 2000). The Na^+ effluence at the root surface and Na^+ transport from root to shoot are mediated by *SOS1* (Tester and Davenport, 2003). Then, the K^+/Na^+ ratio is advanced appropriately in leaves as the significant site for performing metabolic activities. So, the *SOS3/SOS2* complex activates the Na^+/H^+ antiporter promotion and the expression regulation of the *SOS1* gene for its activity (Sánchez-Barrena *et al*., 2005). Mutants lacking in SOS2 and SOS3

exhibit salt-sensitive phenotypes analogous to *SOS1* plants (Zhu, 2001).

This study aimed to reveal how the selected EMS mutants improved salinity tolerance. So, the following objectives were investigated under salt stress:

Evaluation of differences in Na^+/K^+ homeostasis among the mutant rice genotypes and control;

Clarification of some morphological and biochemical traits at different time points and the expression levels of key genes (NHX1 and SOS1) concerning ionic responses and their rules in the defense strategies in the mutant rice genotypes (em_4hs290 and em_4hs84) in comparison with the control rice cultivars;

Revealing the importance of mutation in improving tolerance to salt stress in mutant rice genotypes.

MATERIALS AND METHODS

Experimental Materials Selection

To evaluate salinity tolerance in the mutant genotypes, we first surface-sterilized the seeds of salt-sensitive (IR28) and tolerant (FL478) varieties and two EMS-derived salt-tolerant mutants (*em_4hs290* and *em_4hs84*) in rice (*Oryza sativa, cv. Hashemi*) at Rice Research Institute of Iran (Rasht, Guilan Province, Iran), in 2018-2019. Forty healthy seeds were placed equally on filter paper in a 9-cm-diameter Petri dish. After four days, the germinated seeds were transferred to perforated Styrofoam floats with a net bottom suspended on buckets in a hydroponic system with Yoshida solution (Yoshida and Coronel, 1976) in the greenhouse. Plants were grown in a greenhouse under structured conditions (25 °C, 60% humidity, 16/8 hours light/dark cycle). The nutrient solutions were exchanged every five days. After 14 days, seedlings were grown under normal conditions, then, one compartment of the nutrient solutions was treated with 100 mM NaCl solutions (about ECiw 10 dS m^{-1}) based on the test results to determine the appropriate salt concentration of Hashemi rice (Khazaie *et al*., 2023), while control plants were supplied in a nutrient solution without NaCl (ECiw 0 dS m^{-1}). A part of the samples was immediately frozen in liquid nitrogen at four-time points 6, 24, 48, and 72 h after the onset of salinity treatment, chosen to capture both early and late stress responses, then, kept at −80°C until RNA extraction. Another part of the samples was kept for biochemical, physiological, and growth parameters measurements. Ten seedlings were collected for each time point measurement.

Physiological and Growth Parameters

The root length, and also length of six rice seedlings were measured with a ruler after 14 days of salinity stress. After removing three seedlings of roots, stems and leaves, the fresh weight of each seedling and root was calculated on a scale (±0.001 g). Besides, the seedlings were oven-dried at 40 °C for three days following measurement of dry weights of the seedlings and roots.

Proline Concentration Measurement

Fresh shoot tissues of the rice genotypes were collected at different time points (6, 24, 48, and 72 hours after salinity stress), then, proline content was measured according to the protocol instruction provided by Bates *et al*. (1973). The Proline concentration was estimated by a standard curve (L-proline) and read as micrograms per gram of fresh weight.

Determination of Na^+ and K^+ Content

K^+ and Na^+ concentration in shoot tissues was determined using the method developed by Isaac and Johnson Jr (2019). After drying and grinding the plant samples, each sample was digested on the digestion unit including

a di-acid mixture (20 mL) containing HNO3 and HClO4 acid (9:4) (Turbotherm, Gerhardt analytical systems, Germany) according to the established procedure (Tandon, 1995). The concentration of K^+ and Na^+ samples and the standard solutions were determined by a flame photometer (Systronics FF128).

RNA Extraction and Semi-Quantitative RT-PCR

Samples of 14-day old leaves were collected after 6, 24, 48, and 72 hours for gene expression analysis. Total RNA was extracted by utilizing RNX-plus TM (Synaclone) and measured using Thermo Scientific NanoDrop 2000 (USA). Then, cDNA synthesis was constructed according to the instructions of Thermo Scientific™ Fermentas First Strand cDNA Synthesis Kit. The housekeeping gene UBQ10 in rice (accession no. AT4G05320) was used as the reference gene (Yang *et al*., 2012).

The specific gene primers related to ionic homeostasis were designed by Primer3 Input (version 0.4.0) (Table 1). The Real-Time PCR reactions were performed in the iQ5 (Bio-Rad, Palo Alto, USA), and PCR programs were done as follows: at first, an initial denaturation at 95°C for 4 minutes, then, samples were located in a cycling regime of 45 cycles at 95°C for 30 seconds, 58-60°C for 30 seconds and 72°C for 30 seconds. The quantitative real-time PCR (qRT-PCR) method and data analysis were performed as provided by Pfaffl and colleagues (Pfaffl *et al*., 2002).

Data Analyses

Statistical analysis was performed in a random complete factorial with three repeats using one-way ANOVA, followed by Tukey's HSD test to determine significant differences between treatment groups ($P< 0.05$) through SAS ver9.2 software.

RESULTS

Effects of Salt Stress on Rice Seedling Growth

After treating the two-week old seedlings with 100 mg NaCl, the onset of morphological damage was observed after three days. On the seventh day, morphological changes such as a rolling leaf, whitening of the leaf tip, growth limitation and, finally, death were found in the plants, while the plants grew normally under the control conditions. The length of IR28 and Hashemi seedlings was considerably decreased (about 44.7, and 44.2% reduction, respectively, compared to the control), and the leaves progressively yellowed. Nevertheless, the mutant genotypes (em_4hs290 and em_4hs84) and FL478 were almost unaffected after 3 to 5 days. IR28 and Hashemi genotypes gradually died after 3 to 5 days of salt stress,

Table 1. The applied specific primers for Q Real-time PCR in rice genotypes.

Gene	Primer sequence (5'→3')	Product length (bp)	Melting temperature	Tm (ºC)	NCBI accession number
Ubiquitin10	*F-TGGTCACTAATCAGCCAGTTTGG* *R-CACCACAAATACTTGACCAACAG*	81	60.65 61.01	59	XM_015769228.1
SOS1	*F-ACTTGGACGATGAGCCTGTG* *R-ATTTAGAAGCCGCACACGGA*	98	60.04 60.04	58	XM_015763865.2
OsNHX1	*F-TCCAGCCTCCGGATGCT* *R-ATCAGCGCGTCGTCGAA*	77	60.00 59.46	60	XM_006658017.2

whereas the older leaves of the mutant genotypes and FL478 just started to turn yellow. The mutant genotypes and FL478 were able to grow and produce new leaves seven days after the salinity stress (Figure 1). Thus, the genotype's survival was graded as *em$_4$hs290*> *em$_4$hs84*> *FL478*> Hashemi cultivar> *IR28* (Table 2). These results illustrated that the mutant genotypes and FL478 are more tolerant than Hashemi cultivar and IR28.

Ion Content Changes in Shoots of the Genotypes under Salt Stress

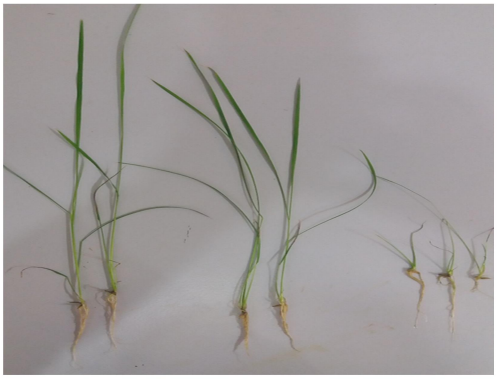
Under the salinity stress, K^+ and Na^+ content and K^+/Na^+ ratio displayed a significant ($P< 0.01$) difference among the mutant genotypes and the control cultivars. Regardless of the type of tissues and genotypes, salinity stress reduced K^+ content. The K^+ content in the leaves of *em$_4$hs290* and *em$_4$hs84* reached from 4.05 to 4.25%, which is higher than IR28 and Hashemi cultivars (2.81 and 3.11%) (Figure 2-a). The Na^+ content in both mutants was 0.23%, lower than that of *IR28* and Hashemi cultivar (0.59 and 0.48%) (Figure 2-b). The Na+ accumulation in leaves and stems of all evaluated genotypes was extremely higher after 72 h stress induction. Plants may develop different approaches to achieve salinity tolerance by adapting via regulation of osmotic adjustment, tissue tolerance adaptation, restriction in Na^+ ion loading and accumulation in tissues, or Na^+ exclusion from the cytosol (Shabala *et al*., 2010; Cuin *et al*., 2011; Shahzad *et al*., 2022). Moreover, K^+/Na^+ ratio in different parts of all genotypes decreased under salinity stress. The K^+/Na^+ ratio in *R28* and Hashemi cultivars (0.22 and 0.15%, respectively) was higher than that of the mutant genotypes (0.051 to 0.054) and FL478 (0.062). The ability of salinity tolerant genotypes to decrease Na^+ net uptake and maintain K^+ uptake triggered desirable K^+/Na^+ ratio in all tissues (Figure 2-c).

Physiological Modulations under Salt Stress

The compatible solute accumulation, such as proline, is one of the most important mechanisms involved in crop plant response to abiotic stresses like drought and salinity (Singh *et al*., 2018). The results illustrated that IR28 and Hashemi cultivars accumulated lower proline in comparison to the mutant genotypes and FL478 (Figure 3-a). After applying the stress, the proline concentration increased in *em$_4$hs290* (0.96) and *em$_4$hs84* (0.77) rather than *IR28* (0.42) as compared to the control plants (Figure 3-a). Therefore, the

Table 2. Effect of salt stress on morphological traits of the mutant rice genotypes and control.

Rice genotypes	Stem length (cm)		Root length (cm)		Stem fresh weight (g)		Root fresh weight (g)		Stem dry weight (g)		Root dry weight (g)	
	control	10 dS	control	10 dS	control	10 dS	control	10 dS	control	10 dS	control	10 dS
Fl478	51.9	24.75	10.5	11	0.46	0.125	0.51	0.185	0.07	0.03	0.02	0.015
IR28	42.5	19	8	6.3	0.405	0.07	0.34	0.115	0.11	0.02	0.03	0.01
Hashemi	48.4	21.5	9.1	7.5	0.43	0.1	0.475	0.125	0.065	0.02	0.02	0.01
em3hs84	55.5	30	12.95	10.75	0.9	0.185	0.835	0.275	0.135	0.03	0.04	0.02
em3hs290	54.2	37.5	13.8	13.2	1.11	0.33	0.81	0.645	0.15	0.06	0.03	0.025



(A) (B) (C)

Figure 1. Growth and developmental status of the studied genotypes under salinity stress. A: The rice genotypes under normal conditions, B: The mutant rice genotypes under salinity stress, and C: *IR28* and Hashemi under salinity stress.

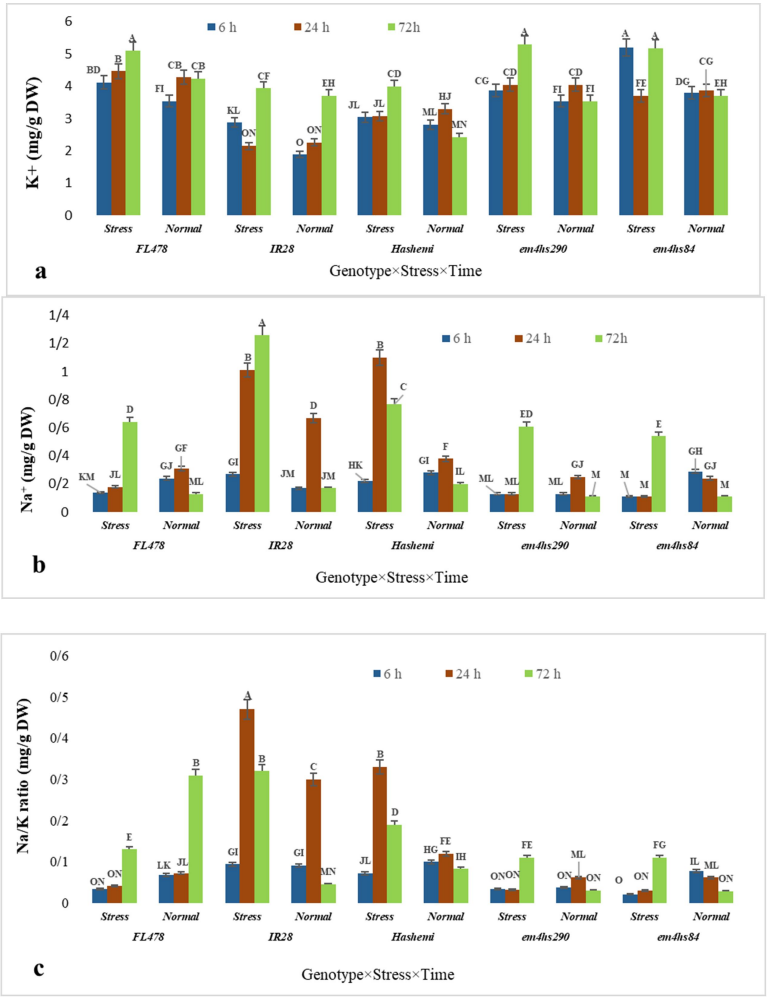


Figure 2. Mean comparison of salt stress effects on cellular ion and mineral accumulation in shoot. Changes in **K+ (a), Na+ (b),** and Na/K ratio (c) in rice genotypes under 100 mM NaCl treatment over time, using Tukey's test ($P< 0.05$).

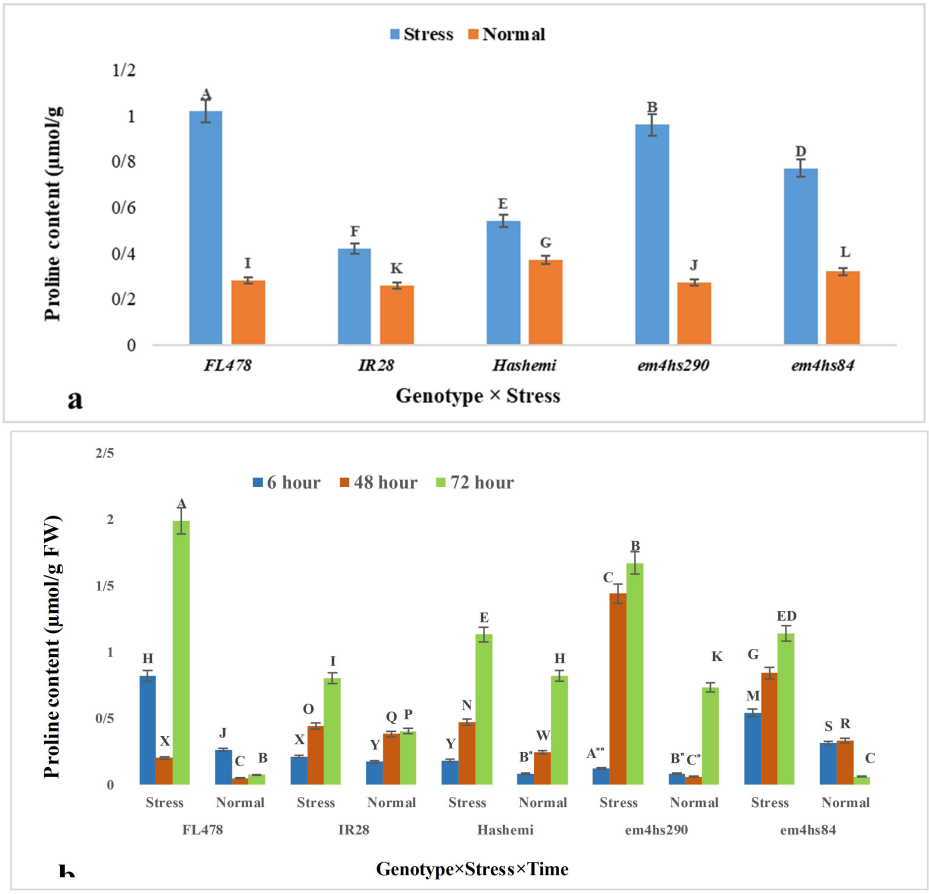


Figure 3. Mean comparison of proline content in the rice genotypes (a), and the interaction of genotype×stress×time (b). Different letters in each rice line show significant differences using Tukey's test at ($P< 0.05$).

tolerant plants accumulated proline to survive against the salt stress, compared to the respective controls (Figure 3-b).

Effect of Salinity Stress on the Expression of Ion Transport-Related Genes

Based on QRT-PCR results, the changes in relative gene expression levels confirmed the relationship between ion transport regulation and salt stress (Figure 4).

The induction of *OsNHX1* and *SOS1* expression was slightly higher in the tolerant genotypes, and reached a peak at 72 h after stress initiation (Figure 4). The expression of *SOS1* increased in *IR28* and Hashemi rice cultivars during 48 and 72 hours. However, the expression of *OsNHX1* elevated in *IR28* at 48 hours after the stress induction (Figure 4-a). The expression of *OsNHX1* and *SOS1* enhanced in tolerant genotypes compared to the susceptible genotype after 6 h of salinity stress. Despite this, after prolonged stress, a significant difference between tolerant and susceptible genotypes was detected. However, the expression levels of *SOS1* increased in *em$_4$hs290* and *FL478* (Figure 4b). The tolerant genotypes also demonstrated early and higher expression in ion transport-related genes (NHX1 and SOS1) compared to the sensitive genotypes. After 6 hours, the expression of genes (OsNHX1 and SOS1) started to increase and presented the most meaningful increase in SOS1 expression, with above a 10-fold increase in shoot tissue after 48 h of salt

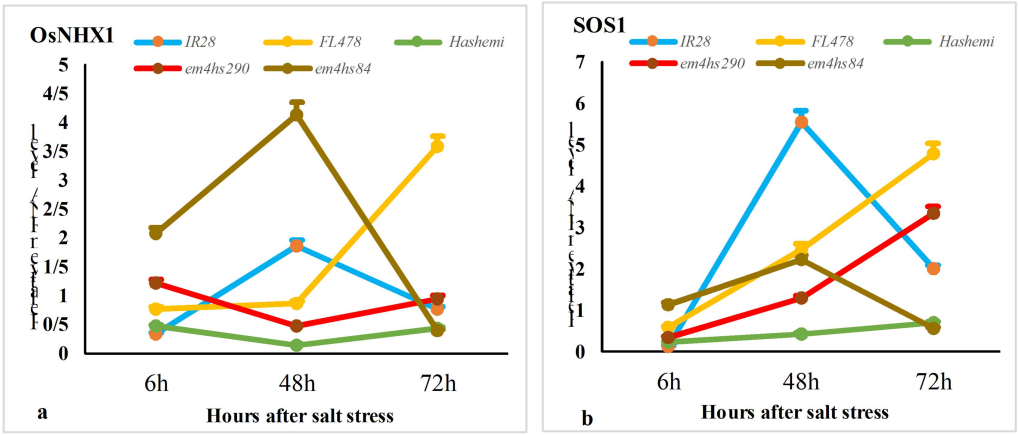


Figure 4. Relative gene expression analysis of the ion transport-related (a: OsNHX1, and b: SOS1) genes by Q real-time PCR among the three biological replicates in the rice genotypes. The studied reference gene was Ubiquitin10. Expression levels of genes in salt-stressed plants were normalized concerning those in non-stressed plants.

stress (Figure 5). After applying salt, the expression levels of *OsNHX1* and *SOS1* genes significantly up-regulated in all genotypes. Remarkably, expression levels of *SOS1* and *OsNHX1* were considerably up-regulated under 48 hours salt treatment in all genotypes. It is generally recognized that Na^+ and K^+ transporter gene families, such as *SOS* and *NHX*, play a significant role in cellular or whole plant Na^+ exclusion, sequestration, and planta movement (El Mahi *et al*., 2019; Martínez-Atienza *et al*., 2007; Shabala and Munns, 2017; Shabala *et al*., 2010).

The results showed that the *SOS1* transcript profile was similar among all genotypes as they all appeared to have an expression peak at 48 h after stress. Notably, a significant difference was not observed in *SOS1* expression among genotypes after 6 h salinity exposure (Figure 4-b). Salt-tolerant genotype (FL478) showed ~5–6-fold higher expression of *SOS1* when compared to Hashemi rice at 48 and 72 hours after stress. Also, *em₄hs290* and *Fl478* reached the highest level of *SOS1* transcript ~3–4-fold transcript levels after 72 h salt treatment. However, *IR28* and *em₄hs84* mutant genotypes showed similar expression patterns under salt stress, nevertheless, the results illustrated a peak of expression level at 48 hours after stress and then showed a sharp decrease after salt treatment at 72 hours. The higher expression of the *SOS1* gene was detected in the salt-resistant genotypes, while the expression decreased considerably in the sensitive genotypes (Figure 4-b).

The results exhibited that the *SOS1* transcript levels were dissimilar among five rice genotypes. *IR28* and *em₄hs84* showed the highest *OsNHX1* expression after salt stress, reaching a peak 48 hours after stress initiation and decreasing at 72 hours after stress. Hence, *em₄hs290* mutant genotype, Hashemi rice and *Fl478* showed a decrease at 48 hours after stress. The results illustrated that a peak in the expression levels of *SOS1* in *FL478* and *em₄hs290* was more intense than in Hashemi cultivar at 48 hours after stress and then showed a sharp increase after salt treatment at 72 hours. Moreover, the resistant genotypes under the control conditions (0 mg NaCl) indicated a higher expression in *OsNHX1* transcript levels compared to the salt-susceptible variety *IR28*.

In general, the mutant tolerant genotypes were able to have different physiological and biochemical responses to salt stress with comparison to control cultivars: *em₄hs290* and *em₄hs84* showed a high K^+ content in leaves, a high proline content, and absorbed more K^+ in the response to salinity. Moreover, the two mutants (em_4hs290, em_4hs84) showed up-regulation of

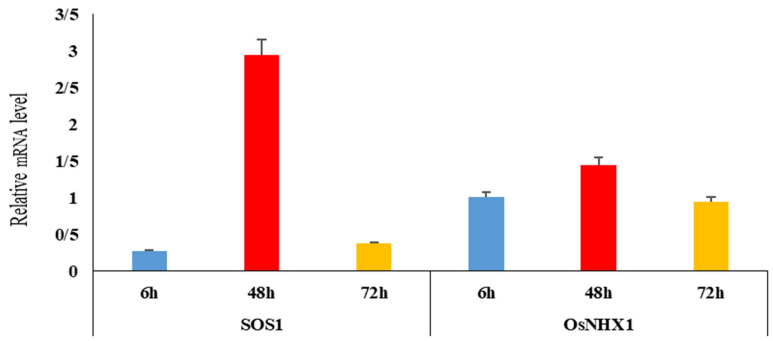


Figure. 5. The relative gene expression levels of *OsNHX1* and *SOS1* genes in the studied genotypes under salinity stress conditions by Q real-time PCR among three biological replicates in the rice genotypes. The applied reference gene was Ubiquitin10.

responsive genes and the inhibition of ion transport.

DISCUSSION

Salt stress restrains crop production through various processes including ionic, osmotic, and oxidative stress. Its direct target is cytoplasmic concentrations via increasing sodium and chloride and disruption of membrane ion transport on cellular processes that could inhibit plant growth and development. The results exhibited that genotype and salinity stress caused meaningful effects on morphological, physiological, and molecular responses. Hence, the results indicated that the mutant genotypes and *FL478* might use different mechanisms in response to stress; because each rice variety could employ one or two salt-tolerance mechanisms (Ganie *et al*., 2019) to decrease the damage of salt stress by adjusting physiological and biochemical mechanisms (Pental, 2019; Rasel *et al*., 2021; Peng *et al*., 2016).

The salinity stress imposed at the seedling stage led to a significant decrease in growth indices in Hashemi rice and *IR28* cultivar under intensive salinity stress, while the responses of mutant genotypes and *FL478* to salinity stress varied, as observed by Zhang *et al*. (2018).

As discussed in the results section, Na^+ content significantly increased at all-time points. This aligns with previous studies by Zhang *et al*. (2022), indicating a common stress response. The results also showed that the mutant genotypes could manage the ion uptake in the shoots by absorbing more K^+, decreasing the Na^+ concentration and the Na^+/K^+ ratio. These results are in accord with the findings of Nakhoda *et al* (2012) and Shahzad *et al* (2022) (Table 3).

The expression of *SOS1* was up-regulated under salinity in tolerant genotypes in leaf tissues, which could be associated with simplifying the exclusion of toxic Na^+ into root apoplast and their ability to maintain a higher K^+/Na^+ ratio of leaves (Figures 2 and 3a) (Shahzad *et al*, 2022). An increase in the relative expression of *OsNHX1* was observed in mutant genotypes and a decrease in IR28 relative expression at 6 h after stress. The high reaction was observed in the tolerant genotypes at the earliest hours after stress, whereas the sensitive genotype response varied with time. Therefore, these results revealed that the sensitive genotype takes a longer time to operate stress-responsible genes, which could be a factor for delayed hemostasis and high damage due to salinity stress. Nevertheless, it has already been demonstrated that the expression patterns of *NHX-type* genes in salt-sensitive and salt-tolerant plants are varied (Hamada

et al., 2001; Gong *et al*., 2005; Zhang *et al*., 2008; Xia *et al*., 2002).

Therefore, the results of the experiments obviously demonstrated that mutant genotypes could perform better than *IR28* and Hashemi rice under salinity level of 10 $dS.m^{-1}$ at early seedling stage. In rice, the salt tolerance in the seedling stage varies with the salinity tolerance during the other growth periods and may not be associated with each other (Jenks *et al*., 2007; Singh *et al*., 2010). For this reason, we need to characterize the rice salinity tolerance during the entire growth period in the field. These results contribute to understanding salinity tolerance mechanisms in rice by highlighting the role of *SOS1* and *NHX1* gene expression in Na^+ and K^+ homeostasis. However, further research is needed to elucidate these pathways fully. The results of this research and other studies illustrated that breeding by mutation method has the potential to create new cultivars with desirable morphological characteristics. This study highlights the potential of Hashemi rice mutants in improving salinity tolerance. Considering the results from all of the experiments, Ethyl Methanesulfonate (EMS) effectively induced variation in salt tolerance in Hashemi rice and was a successful method for developing salt-tolerant varieties and yield sustainability in rice.

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بهبود شاخص‌های تحمل به شوری و تنظیم هموستازی یون‌های Na^+ و K^+ در موتانت‌های برنج هاشمی

لیلا خزائی، و رضا شیرزادیان خرم‌آباد

چکیده

تنش شوری یک تهدید بزرگ زیست محیطی برای توسعه عملکرد محصول است. از این رو، توسعه هر طرح اصلاحی نیاز به درک اولیه فیزیولوژی و ژنتیک سلول‌های تحت تنش شوری دارد. در این پژوهش، پروفایل بیان ژنهای موثر بر هموستازی یونی شامل حساسیت بیش از حد نمک (SOS1) و آنتی پورتر Na^+/H^+ واکوئلی (NHX1) همراه با اندازه‌گیری محتوای یون و محتوای پرولین در ژنوتیپ‌های جهش یافته متحمل در موسسه تحقیقات برنج کشور در سال‌های ۱۳۹۷-۱۳۹۸ مورد بررسی قرار گرفت. به‌منظور بررسی این واقعیت‌ها، ژنوتیپ‌های جهش‌یافته متحمل (*em4hs290* و *em4hs84*) همراه با رقم والد هاشمی، IR28 (حساس) و FL478 (مقاوم) تحت تنش ۱۰۰ میلی‌مولار NaCl قرار گرفتند. بر اساس نتایج شاخص‌های رشد، طول ساقه رقم هاشمی و IR28 به طور قابل‌توجهی حدود ٤٤/٧ درصد و ٤٤/٢ درصد نسبت به شرایط شاهد کاهش داشتند و برگ‌ها به تدریج زرد شدند. نتایج نشان داد که محتوای پرولین و نسبت K^+ و K^+/Na^+ در ژنوتیپ‌های متحمل حدود ۲ تا ۳ برابر بیشتر از ژنوتیپ‌های حساس با قرار گرفتن در معرض تنش شوری افزایش یافت. همچنین، میزان کل بیان OsNHX1 و SOS1 در ارقام متحمل بیشتر از ارقام حساس بود. بنابراین، بیان بالای ژن‌های مرتبط با گروه یونی (OsNHX1 و SOS1) در برگ ژنوتیپ‌های جهش یافته *em4hs84*و*em4hs290* تجمع املاح سازگار به‌طور قابل‌توجهی افزایش می‌دهد و هموستازی یونی را ارتقاء می‌دهد و احتمالاً تنش را مهار می‌کند. الگوی متغیر بیان ژن‌های مورد مطالعه در دو ژنوتیپ جهش‌یافته متحمل به نمک (*em4hs290* و *em4hs84*) و والد هاشمی نشان داد که جهش می‌تواند توانایی ژنوتیپ جهش‌یافته متحمل به نمک را در استفاده از هموستاز یونی و اسمزی در پاسخ به تنش شوری تغییر دهد. به‌طور کلی، این ژنوتیپ‌های جهش‌یافته متحمل را می‌توان برای توسعه واریته‌های متحمل به شوری در برنامه های اصلاحی برنج انتخاب کرد که می‌تواند پایداری تولید را به همراه داشته باشد.